Mouse Embryoid Bodies (EBs) as a Source of Renal Tubular Progenitor Cells

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Previous studies from this laboratory have shown that human renal progenitor cells could be isolated from microdissected fetal kidneys and maintained through multiple passages in an undifferentiated state in serum-free, G401-conditioned media (CM) and stimulated to differentiate into epithelial foci by withdrawal of G401-CM and addition of 10% serum and/or collagen matrix (Burrow and Wilson, 1993). Subsequent, similar studies of mouse fetal kidneys showed that although progenitor cells could be isolated and induced to differentiate, cell numbers, proliferation, and survival of undifferentiated progenitors were limiting. With a view to examine renal stem cell development for potential therapeutic uses, we have utilized the mouse embryonic stem (ES) cell/ embryoid body (EB) system (Keller, et al., 1993; Keller, 1995; Levin, et al., 2003). Totipotent ES cells can be cultured under conditions to give rise to EB aggregates that contain a variety of ectodermal, mesodermal, and endodermal lineages. Importantly, culture for different lengths of time in the absence of Leukemia Inhibitory Factor (LIF) or serum and in the presence of specific growth factors and/or feeder cell layers has been shown to enrich EBs for specific cell lineages (Kubo, et al., 2004).

Here we have used Rosa-LacZ- ES cells with GFP knocked into the functional Brachyury locus to generate EBs in culture. Following FACS sorting of GFP+ and GFP- populations and RT-PCR marker analysis of brachyury, WT-1, cadherin-11, Pax-2, and wnt-4, we concluded that EBs contained renal progenitors and that these were maximally enriched after 4 days in culture. Additional optimization studies showed that low concentrations of activin in serum-free media also promoted renal progenitor differentiation. To determine whether these EB-derived renal progenitor cells were functional *in vivo*, FACS-purified, LacZ+GFP+ populations were injected into the left kidneys of eight newborn mouse pups under light anesthesia without surgical intervention and the kidneys were examined after 2 weeks of post-natal development. Right kidneys were not injected and were also examined as controls. Anti-β–galactosidase immunostaining showed dramatic incorporation of EB-derived progenitors into tubular profiles of the entire cortical region of all injected kidneys. No staining was seen in uninjected contralateral kidneys. No evidence of inflammation or teratoma induction was seen. Immunostaining of serial sections using antibodies against renal tubule segment specific markers including aquaporin-1, aquaporin-2, calbindin, and TAMM-Horsfall protein suggest that LacZ+ GFP+ EB-derived progenitors were incorporated into renal proximal tubules.

We conclude that mouse ES cell-derived EBs can be induced to generate renal progenitor cells by optimization of length of time in culture and a specific growth factor environment, that these cells are functional and differentiate and incorporate into renal proximal tubule epithelia in *vivo*. The therapeutic potential of these cells is being tested.

References

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